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New species and a new combination in the *Hyphopichia* and *Yarrowia* yeast clades

Cletus P. Kurtzman
Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, 1815 N. University Street, Peoria, Illinois 61604, USA; (e-mail: Kurtzman@ncaur.usda.gov)

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Abstract

Three new species of *Candida* and a new combination in the genus *Hyphopichia* are proposed from phylogenetic analysis of nucleotide divergence in domains D1/D2 of the large subunit (26S) rDNA. The new taxa and their type strains are the following: *Candida bentonensis* sp. nov. (NRRL YB-2364, CBS 9994), *Candida hispaniensis* sp. nov. (NRRL Y-5580, CBS 9996), *Candida pseudorhagii* sp. nov. (NRRL YB-2076, CBS 9998) and *Hyphopichia heimii* comb. nov. (NRRL Y-7502, CBS 6139), basionym *Pichia heimii* Pignal. Phylogenetic analysis placed *C. pseudorhagii* and *H. heimii* in the *Hyphopichia* clade whereas *C. bentonensis* and *C. hispaniensis* are members of the *Yarrowia* clade.

Introduction

The ascosporic yeast genera *Hyphopichia* and *Yarrowia* were each described from a single, phenotypically distinct species. Von Arx and van der Walt (1976) placed *Pichia burtonii* in their newly created genus *Hyphopichia* using the combined characters of heterothallism, septate hyphae and denticulate conidiogenous cells as primary descriptors. Various other yeasts, as well as some dimorphic euascomycetes, share these same characters and the genus *Hyphopichia* has not been generally accepted because of the lack of unique characters (Kurtzman 1998). Similarly, the genus *Yarrowia* (van der Walt and von Arx 1980) was constructed for the previously described ascosporic state of *Candida lipolytica* using as defining characters true hyphae, denticulate conidiogenous cells, variability of ascospore morphology and the presence of coenzyme Q-9 in the electron transport system. Despite the uncertainty of these phenotypic descriptors, *Hyphopichia* and *Yarrowia* proved to be well separated from other genera when phylogenetically analyzed from rDNA sequences (Kurtzman and Robnett 1998a). This is in contrast to many other genera that have been defined from phenotype, such as *Wingea* (van der Walt 1967), *Kluyveromyces* (van der Walt 1956) and *Pichia* (Kurtzman 1998), which are not phylogenetically well circumscribed (Kurtzman and Robnett 1998a, 2003).

Because *Hyphopichia* and *Yarrowia* appear phylogenetically separate from one another as well as from other yeasts, do they occupy unique ecological niches that have been responsible for their genetic isolation as monotypic genera, or have neighboring species not yet been discovered? In the present study, new species closely related to *H. burtonii* and *Y. lipolytica* are described. Recognition of these new species resulted from

rDNA comparisons and demonstrates that the perceived genetic isolation of *H. burtonii* and *Y. lipolytica* is an artifact of failure to recognize genetic relationships among species from phenotype. Consequently, it seems likely that many lineages with few species will be expanded through a combination of increased isolation of novel yeasts and their characterization from gene sequences.

Materials and methods

Organisms and physiological tests

Strains of the proposed new species and their sources of isolation are given in Table 1. The strains are maintained in the Agricultural Research Service (ARS) Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, Illinois, USA. The composition of culture media used in this study, methods for preparing and assessing fermentation and assimilation tests, and the procedure for conducting the Diazonium Blue B (DBB) test were given by Yarrow (1998). The dye reagent for the DBB test was Fast Blue B salt (Sigma D9805), and determinations were made on cultures grown for both 1 and 3 weeks.

DNA isolation, sequencing and sequence analysis

Procedures for DNA isolation and sequencing of domains D1/D2 of the large subunit rDNA were previously given (Kurtzman and Robnett 1998a). Both strands of the DNAs compared were sequenced with the ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) using either an ABI 3100 or an ABI 3730 automated DNA sequencer according to manufacturer's instructions. Following visual alignment of sequences, estimates of phylogenetic relatedness among species were determined using the maximum parsimony and neighbor-joining programs of PAUP* 4.063a (Swofford 1998). The datasets included several regions of uncertain nucleotide alignment. Because of this, phylogenetic analyses were conducted using the apparent best alignment of the complete D1/D2 sequences as well as with a dataset in which regions of ambiguous alignment

Table 1. Sources of the strains compared.

Species	Strain number[a,b]		GenBank[c] Accession No. D1/D2 26S rDNA	Source
	NRRL	CBS		
Candida bentonensis	YB-2364^T	9994	AY789653	Apple cider, Benton, Illinois
Candida hispaniensis	Y-5579	9995		Larva of *Spondylus buprestoides*, El Ventorrillo, Spain
	Y-5580^T	9996	AY789654	Same as Y-5579
Candida pseudorhagii	YB-1234	9997	AY789655	Insect frass, unidentified conifer, Lake Wabatongushi, Ontario, Canada
	YB-1998			Insect frass, jack pine (*Pinus banksiana*), Duluth, Minnesota, USA
	YB-2003			Insect frass, longleaf pine (*Pinus palustris*), Wilma, Florida, USA
	YB-2076^T	9998	AY789656	Insect frass, shortleaf pine (*Pinus echinata*), Salem, Missouri, USA
	YB-3075			Insect frass, white pine (*Pinus strobus*), Big Lake, Wisconsin, USA
	YB-3093			Insect frass, dead fir (*Abies* sp.), Big Lake, Wisconsin, USA
Candida rhagii	Y-2594^T	4237	U45729	Cerambycid beetle (*Harpium inquisitor*), Germany
Hyphopichia heimii	Y-7502^T	6139	U45713	Insect-invaded wood, Equatorial Africa

[a] NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
[b] T, Type strain.
[c] Strains Y-5579 and Y-5580 have the same D1/D2 rDNA nucleotide sequence. Strains YB-1998 YB-2003, YB-2076, and YB-3075 have identical D1/D2 sequences; strains YB-1234 and YB-3093 have identical D1/D2 sequences, but differ from the preceding group at one nucleotide position.

were removed. Bootstrap support for phylogenetic trees was determined from 1,000 replications.

Results and discussion

Hyphopichia clade

Phylogenetic analysis of D1/D2 26S rDNA sequences from the work of Kurtzman and Robnett (1998a), with inclusion of more recent submissions to GenBank, demonstrated that the genus *Hyphopichia*, as represented by *H. burtonii*, is phylogenetically isolated from other yeast genera. The *Hyphopichia* clade includes *Candida fennica*, which is sister to *H. burtonii*, *C. gotoi*, *C. homilentoma*, and *C. rhagii*, as well as *Pichia heimii* and NRRL YB-2076, a new species of *Candida* closely related to both *C. rhagii* and *P. heimii* (Figure 1). *Candida* sp. nov. NRRL YB-2076 differs from each of the preceding two species at five noncontiguous nucleotide positions located in both the D1 and D2 domains. *P. heimii* and *C. rhagii* differ from one another at just two nucleotide positions and may represent a teleomorph–anamorph connection. As a result of the molecular comparisons, the following two taxa are proposed.

Hyphopichia heimii (Pignal) Kurtzman comb. nov.

Basionym: *Pichia heimii* Pignal. Antonie van Leeuwenhoek 36:525. 1970.

Type strain: NRRL Y-7502 (CBS 6139).

Latin diagnosis of *Candida pseudorhagii* Kurtzman sp. nov.

In agaro malti post dies 3 ad 25 °C, cellulae vegetativae globosae (2.0–5.2 μm) aut elongatae (1.8–5.0 × 2.0–8.5 μm), singulae vel binae. In agaro morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, nitens, butyrosum; centrum

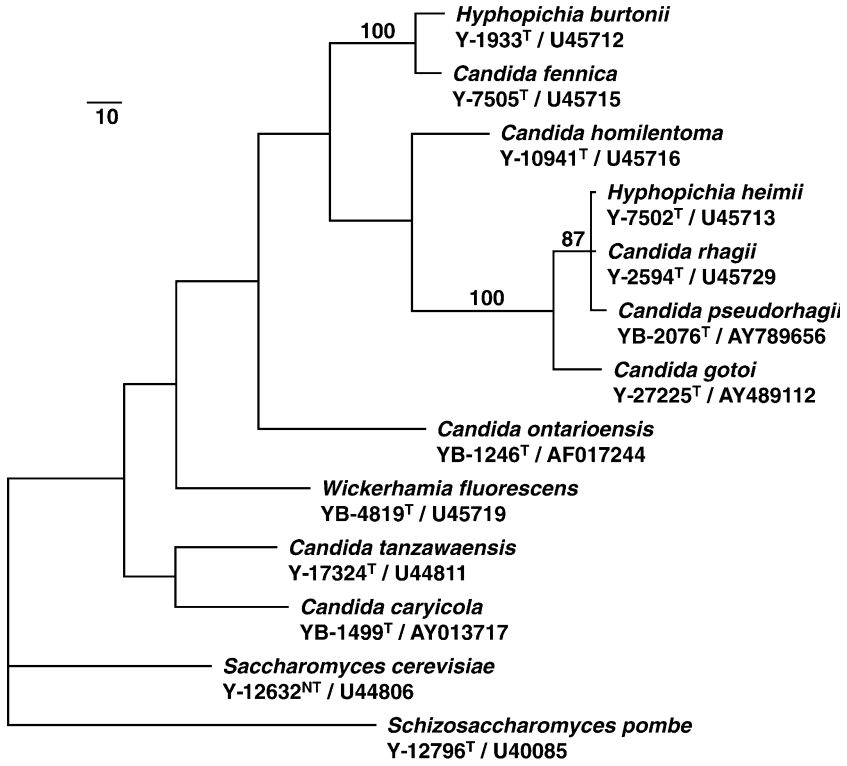


Figure 1. Phylogenetic tree showing placement of *Candida pseudorhagii* and *Hyphopichia heimii* among members of the *Hyphopichia* clade as represented by 1 of 8 most parsimonious trees derived from maximum parsimony analysis of domains D1/D2 26S rDNA. Tree length – 540, consistency index (CI) – 0.683, retention index (RI) – 0.600, rescaled consistency index (RC) – 0.410, homoplasy index (HI) – 0.317. Branch lengths are indicated by the marker bar, and numbers at nodes are bootstrap values determined from 1,000 replications. Frequencies under 50% are not given. *Schizosaccharomyces pombe* was the outgroup species in the analysis.

coloniae sublatum; margo undulata. Pseudohyphae et hyphae verae fiunt. Ascosporae non fiunt.

Glucosum, galactosum, sucrosum, raffinosum (infirme) et trehalosum fermentantur. Maltosum et lactosum non fermentantur. Assimilantur glucosum, galactosum, L-sorbosum (variabile), sucrosum, maltosum, cellobiosum, trehalosum, raffinosum, melezitosum, D-xylosum, L-arabinosum, D-arabinosum (variabile), D-ribosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, ethanolum, glycerolum, erythritolum, ribitolum, D-mannitolum, D-glucitolum, methyl-α-D-glucosidum, salicinum, DS-gluconatum, 2-keto-D-gluconatum, DL-acidum lacticum (variabile), acidum succinicum, hexadecanum et cadaverinum. Non assimilantur lactosum, melibiosum, inulinum, amylum solubile, methanolum, galactitolum, 5-keto-D-gluconatum, saccharatum, acidum citricum, inositolum et potassii nitras. Vitaminae externae ad crescentiam necessariae non sunt. Amylum non formatur; gelatinum liquescit (infirme), in cycloheximido 100 μg/ml non crescit. Pellicula fit. Augmentum variabile fit in temperatura 37 °C (variabile).

Typus: Holotypus NRRL YB-2076 (CBS 9998) lyophilus. Cultura isolata a dejectu coleopterorum in Pinus echinata, Salem, Missouri, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, Illinois, USA.

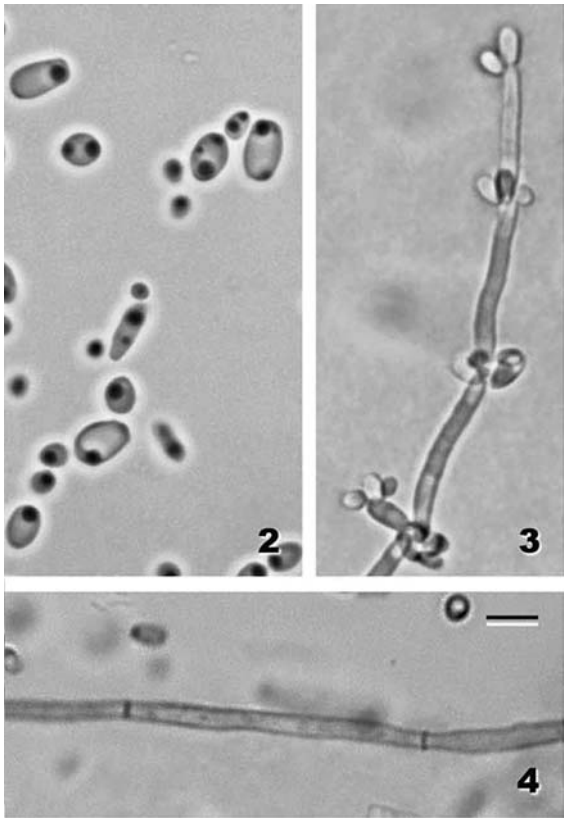


Figure 2–4. Candida pseudorhagii NRRL YB-2076. (2) Budding cells, ME agar, 3 days, 25 °C. (3) Pseudohyphae from under the coverglass of a Dalmau plate culture, yeast morphology agar, 7 days, 25 °C. (4) True (septate) hypha, YM agar, 29 days, 25 °C. Bar = 5 μm for all figures.

Description of Candida pseudorhagii

Growth on 5% malt extract (ME) agar
After 3 days at 25 °C, yeast cells are spherical (2.0–5.2 μm) to elongate (1.8–5.0 × 2.0–8.5 μm) and single or infrequently in pairs (Figure 2). Budding is multilateral. Moderately branched pseudohyphae are present, but true hyphae were not observed on this medium. Growth on 5% ME agar is white, semi-glistening and butyrous with a fine pseudomycelial fringe.

Dalmau plate culture on yeast morphology agar
After 7 days at 25 °C, growth under the coverglass is sparse to moderate with abundant pseudohyphae bearing blastoconidia. Pseudohyphae may be sparsely or abundantly branched (Figure 3). True hyphae were not observed in Dalmau plate culture, but an outgrowth of true hyphae formed in a YM agar culture of NRRL YB-2076 after one month at 25 °C (Figure 4). Aerobic growth on morphology agar is white, dull-glistening, slightly raised with a flat center, and with finely to moderately lobate margins having a narrow pseudomycelial fringe. The colony texture is butyrous, and cultures produce a faint ester-like odor.

Examination for ascospore formation
The six strains of *C. pseudorhagii* were grown alone and as mixtures on YM and 5% ME agar media at 15 and 25 °C and examined microscopically at 3–5-day intervals for 6 weeks. Neither conjugations nor ascospores were observed.

Fermentation, assimilation and other growth tests
Reactions on the various tests are given in Table 2. Thin pellicles formed on the surface of stationary

liquid media and moderate amounts of riboflavin were produced after 1 week of incubation.

Type
NRRL YB-2076 (CBS 9998) is designated as the type strain and is preserved as a lyophilized preparation in the ARS Culture Collection, Peoria, Illinois, USA. The strain was isolated from insect frass in a shortleaf pine (*Pinus echinata*) growing near Salem, Missouri, USA by L.J. Wickerham in July 1950, and has been preserved in the lyophilized state since isolation.

Etymology
The species name *pseudorhagii* refers to the close relatedness of this species with *C. rhagii*.

Examination for ascospore formation in mixtures of C. pseudorhagii, C. rhagii and H. heimii

Ascosporulation has not been observed in *H. heimii* since its description, which clearly illustrated hat-shaped ascospores formed in deliquescent asci (Pignal 1970). Because of their close relatedness, *H. heimii* and *C. rhagii* were examined alone and in mixtures with each other and in mixtures with all *C. pseudorhagii* strains. Conditions were as described above for ascosporulation tests. None of the strains, alone or in mixtures, showed conjugations or ascospore formation. Either conditions for ascosporulation have not been fulfilled or all strains represent the same mating type.

Phenotypic separation of species in the Hyphopichia clade

The seven species in the *Hyphopichia* clade (Figure 1), most of which have been isolated from the frass of insect tunnels, ferment and assimilate many of the compounds included in standard identification tests (Nakase and Suzuki 1997; Kurtzman and Fell 1998; Kurtzman and Robnett 1998b; Kurtzman 2001) and for this reason, *H. burtonii* cannot be reliably separated from *C. fennica*, and *C. pseudorhagii* and *C. rhagii* cannot be separated from each other. However, the clade can be separated into two groups on the basis of soluble starch assimilation, which is positive for *H. burtonii*, *C. fennica*, *C. homilentoma* and *C. gotoi* and negative for *H. heimii*, *C. pseudorhagii* and *C. rhagii*. *C. gotoi* can be separated from members of its group by the assimilation of galactitol, and *C. homilentoma* can be separated from *H. burtonii* and *C. fennica* by the assimilation of L-rhamnose. Of the second group, *H. heimi* assimilates galactitol. As noted earlier, *H. heimii* and *C. rhagii* differ by only two nucleotides in domains D1/D2 of 26S rDNA and may be conspecific.

Yarrowia clade

Yarrowia lipolytica is the only ascosporic member of its clade. As determined from phylogenetic analysis of nucleotide sequences from domains D1/D2 26S rDNA, other species in this clade include *Aciculoconidium aculeatum*, *C. galli* (Péter et al. 2004), *C. incommunis*, and the two new species of *Candida* that are proposed here. From maximum parsimony (MP) analysis, bootstrap support for the clade is 99% (Figure 5). Analysis by neighbor-joining gave a phylogenetic tree congruent with MP analysis, as did an analysis in which all areas of questionable nucleotide alignment were removed.

Latin diagnosis of Candida bentonensis *Kurtzman sp. nov.*

In agaro malti post dies 3 ad 25 °C, cellulae vegetativae ellipsoideae aut elongatae (1.5–4.0×2.5–10.0 μm), singulae vel binae. In agaro morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, hebes, butyrosum; margo undulata. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt.

Glucosum (infirme) fermentatur. Galactosum, sucrosum, maltosum, lactosum, raffinosum et trehalosum non fermentantur. Assimilantur glucosum, galactosum (infirme), L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum, D-xylosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, ethanolum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, methyl-α-D-glucosidum, salicinum, D-gluconatum, 2-keto-D-gluconatum, 5-keto-D-gluconatum, DL-acidum lacticum, acidum succinicum, acidum citricum, inositolum, hexadecanum, potassii nitras et cadaverinum. Non

Table 2. Physiological characteristics of the three new *Candida* species described.

Physiological test	Reaction of [a]			Physiological test	Reaction of		
	C. bentonensis	*C. hispaniensis*	*C. pseudorhagii*		*C. bentonensis*	*C. hispaniensis*	*C. pseudorhagii*
Fermentation							
Glucose	w	–	+	Lactose	–	–	–
Galactose	–	–	+	Raffinose	–	–	w
Sucrose	–	–	+	Trehalose	–	–	+
Maltose	–	–	–				
Assimilation							
Glucose	+	+	+	Methanol	–	–	–
Galactose	w	–	+	Ethanol	+	v	+
L-Sorbose	+	w	v	Glycerol	+	+	+
Sucrose	+	–	+	Erythritol	–	–	+
Maltose	+	–	+	Ribitol	+	–	+
Cellobiose	+	–	+	Galactitol	–	–	–
Trehalose	+	+	+	D-Mannitol	+	+	+
Lactose	–	–	–	D-Glucitol	+	+	+
Melibiose	–	–	–	Methyl-α-D-glucoside	+	–	+
Raffinose	–	–	+	Salicin	+	–	+
Melezitose	+	–	+	D-Gluconate	+	–	+
Inulin	–	–	–	2-Keto-D-gluconate	+	–	+
Soluble starch	–	–	–	5-Keto-D-gluconate	+	–	–
D-Xylose	+	–	+	Saccharate	–	–	–
L-Arabinose	–	–	+	DL-Lactate	+	+	–, w
D-Arabinose	–	–	v	Succinate	+	+	+
D-Ribose	–	–	+	Citrate	+	–	–
L-Rhamnose	–	–	+	Inositol	+	–	–
D-Glucosamine	+	–	+	Hexadecane	+	+	+
N-acetyl-D-glucosamine	+	–	+				
Nitrogen sources							
Nitrate	+	–	–	Cadaverine	+	+	+
Additional growth tests							
Vitamin-free medium	–	–	+	Gelatin liquefaction	–	+	w
10% NaCl/5% glucose	w	–	+	Starch formation	–	–	–
Cycloheximide, 10 μg/ml	+	+	–	Growth at 37 °C	+	v	v
Cycloheximide, 100 μg/ml	+	+	–	Diazonium blue B	–	–	nd

[a] –, negative; +, positive; w, weak; v, variable, i.e., + or –; nd, not determined. Reactions determined from the strains listed in Table 1.

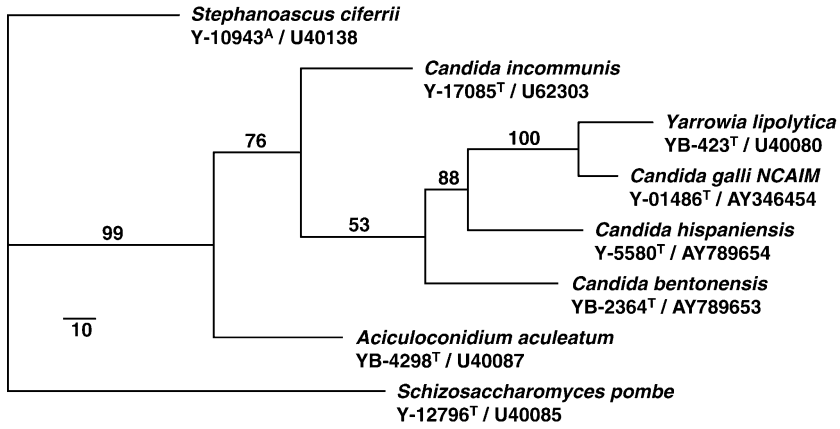


Figure 5. Phylogenetic tree placing *Candida bentonensis* and *C. hispaniensis* among species of the *Yarrowia* clade as represented by 1 of 2 most parsimonious trees derived from maximum parsimony analysis of domains D1/D2 26S rDNA. Tree length – 493, CI – 0.844, RI – 0.601, RC – 0.507, HI – 0.156. Branch lengths are indicated by the marker bar and numbers at nodes are bootstrap values determined from 1000 replications. Frequencies under 50% are not given. The outgroup species for the analysis was *Schizosaccharomyces pombe*.

assimilantur lactosum, melibiosum, raffinosum, inulinum, amylum solubile, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, methanolum, erythritolum, galactitolum et saccharatum. Vitaminae externae ad crescentiam necessariae sunt. Amylum non formatur, gelatinum non liquescit, in cycloheximido 100 μg/ml crescit. Pellicula fit. Augmentum fit in temperatura 37 °C.

Typus: Holotypus NRRL YB-2364 (CBS 9994) lyophilus. Cultura isolata vino malis confecto in Benton, Illinois, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, Illinois, USA.

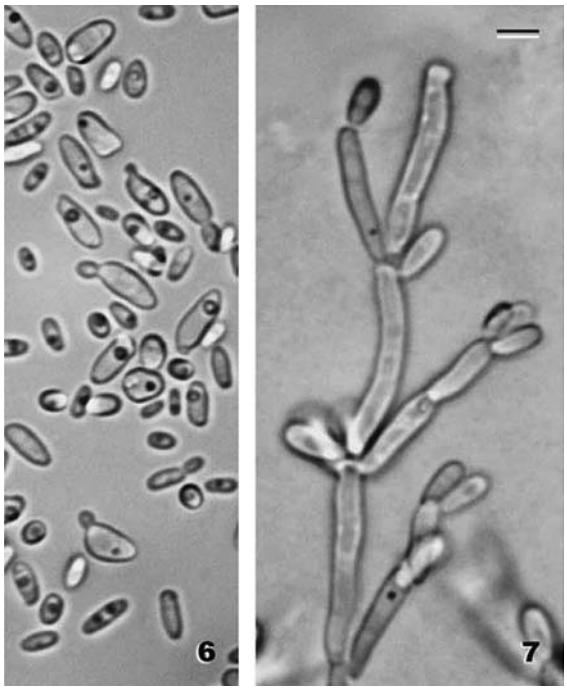
Description of Candida bentonensis

Growth on 5% malt extract agar

After 3 days at 25 °C, yeast cells are ellipsoidal to elongate (1.5–4.0×2.5–10.0 μm) and single or infrequently in pairs (Figure 6). Budding is multilateral. Growth is white, semi-glistening and butyrous.

Dalmau plate culture on yeast morphology agar

After 7 days at 25 °C, growth under the coverglass is moderate with abundant pseudohyphae bearing elongate blastoconidia (Figure 7). True hyphae were not formed. Aerobic growth is white with a dull surface and butyrous in texture. Colonies are raised with a slightly depressed center. Margins are finely and irregularly lobed.



Figures 6–7. *Candida bentonensis* NRRL YB-2364. (6) Budding cells, ME agar, 3 days, 25 °C. (7) Pseudohyphae from under the coverglass of a Dalmau plate culture, yeast morphology agar, 7 days, 25 °C. Bar = 5 μm for both figures.

Examination for ascospore formation

Cultures of NRRL YB-2364 were incubated at 15 and 25 °C on YM, 5% ME and McClary's acetate agar media and microscopically examined at

weekly intervals for 3 months, but neither conjugations nor ascospores were observed.

Fermentation, assimilation and other growth tests
Reactions are given in Table 2. Incomplete pellicles formed on the surface of stationary liquid media. The DBB test was negative.

Type
NRRL YB-2364 (CBS 9994) is designated as the type strain and is preserved as a lyophilized preparation in the ARS Culture Collection, Peoria, Illinois, USA. The strain was isolated from apple cider purchased in Benton, Illinois USA by L.J. Wickerham in October 1950, and had been preserved in the lyophilized state since isolation.

Etymology
The species name *bentonensis* refers to the isolation from cider originating in Benton, Illinois.

Latin diagnosis of Candida hispaniensis *Kurtzman sp. nov.*

In agaro malti post dies 3 ad 25 °C, cellulae vegetativae globosae (3.0–6.0 μm) aut elongatae (2.0–4.0×4.0–9.0 μm), singulae vel binae. In agaro morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, hebes, butyrosum; centrum coloniae convexus; margo undulata. Pseudohyphae raro fiunt; hyphae verae non fiunt. Ascosporae non fiunt.

Glucosum, galactosum, sucrosum, maltosum, lactosum, raffinosum et trehalosum non fermentantur. Assimilantur glucosum, L-sorbosum (infirme), trehalosum, ethanolum (variabile), glycerolum, D-mannitolum, D-glucitolum, DL-acidum lacticum, acidum succinicum, hexadecanum et cadaverinum. Non assimilantur galactosum, sucrosum, maltosum, cellobiosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, erythritolum, ribitolum, galactitolum, methyl-α-D-glucosidum, salicinum, D-gluconatum, 2-keto-D-gluconatum, 5-keto-D-gluconatum, saccharatum, acidum citricum, inositolum et potassii nitras. Vitaminae externae ad crescentiam necessariae sunt. Amylum non formatur, gelatinum liquescit, et in cycloheximido 100 μg/ml crescit. Pellicula fit. Augmentum fit in temperatura 37 °C (variabile).

Typus: Holotypus NRRL Y-5580 (CBS 9996) lyophilus. Cultura isolata a larva ex Spondylus buprestoides, El Ventorrillo, Hispania. Deposita in Collectione Culturarum ARS (NRRL), Peoria, Illinois, USA.

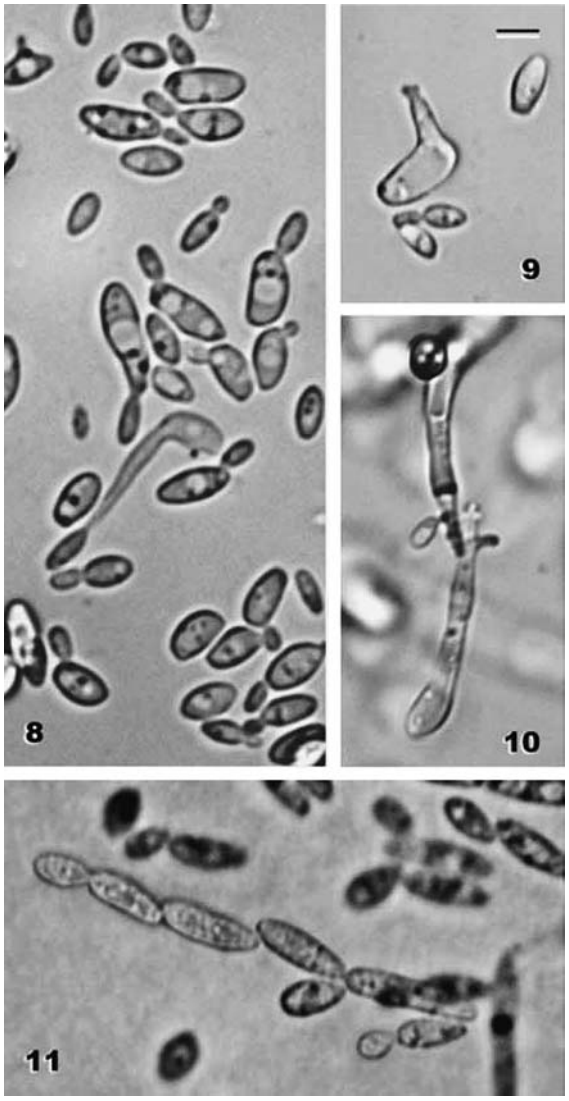
Description of Candida hispaniensis

Growth on 5% malt extract agar
After 3 days at 25 °C, yeast cells are single or in pairs, show multilateral budding and are spherical (3.0–6.0 μm) to elongate (2.0–4.0×4.0–9.0 μm) (Figure 8). Additionally, some of the cells show tapered outgrowths that form blastoconidia and these outgrowths may become quite long. The ends of the outgrowths are often denticulate and bear blastoconidia on the denticles (Figures 9 and 10). Growth is white, semi-glistening and butyrous.

Dalmau plate culture on yeast morphology agar
After 7 days at 25 °C, growth under the coverglass is sparse with 'tree-like' outgrowths of undifferentiated cells (Figure 11). Pseudohyphae and true hyphae are not present. However, some pseudohyphal outgrowths were detected on YM agar after 3 months at 25 °C. Aerobic growth on morphology agar is white with a dull surface, low convex, and with finely lobate margins. The texture is butyrous.

Examination for ascospore formation
Cultures of NRRL Y-5579 and NRRL Y-5580, singly and as mixtures, were incubated at 15 and 25 °C on YM, 5% ME and McClary's acetate agar, and microscopically examined at weekly intervals for 3 months, but neither conjugations nor ascospores were observed.

Fermentation, assimilation and other growth tests
Reactions are given in Table 2. Pellicles formed on the surface of stationary liquid media. NRRL Y-5579 and NRRL Y-5580 gave a negative DBB reaction after 1 and 3 weeks of growth.



Figures 8–11. Candida hispaniensis NRRL Y-5580. (8) Budding cells, YM agar, 1 day, 25 °C. (9) Cell with conidiogenous protuberance, conditions as in *Fig. 8*. (10) Cells with protuberances showing conidiogenous denticles, ME agar, 3 days, 25 °C. (11) Sparingly developed pseudohypha, from under the coverglass of a Dalmau plate culture, yeast morphology agar, 7 days, 25 °C. Bar = 5 μm for all figures.

Type

NRRL Y-5580 (CBS 9996) is designated the type strain and is preserved as a lyophilized preparation in the ARS Culture Collection, Peoria, Illinois, USA. The strain was isolated in April 1960 by Juan Santa Maria from a larva of *Spondylus buprestoides* collected in a conifer forest near El Ventorrillo, Spain, and sent to L.J. Wickerham, NCAUR, Peoria, Illinois.

Etymology

The species name *hispaniensis* denotes that the species originated in Spain.

Phenotypic separation of species in the Yarrowia clade

The six known species of the *Yarrowia* clade (Figure 5) can be separated from one another using the following key, which is based on standard growth responses (Kurtzman and Fell 1998; Péter et al. 2004).

Key to species of the *Yarrowia* clade

1.	a. Erythritol assimilated	2
	b. Erythritol not assimilated	4
2(1).	a. Melezitose assimilated	*C. incommunis*
	b. Melezitose not assimilated	3
3(2).	a. *N*-acetyl-D-glucosamine assimilated	*Y. lipolytica*
	b. *N*-acetyl-D-glucosamine not assimilated	*C. galli*
4(1).	a. Melezitose assimilated	5
	b. Melezitose not assimilated	*C. hispaniensis*
5(4).	a. D-Xylose assimilated	*C. bentonensis*
	b. D-Xylose not assimilated	*A. aculeatum*

In contrast to *Hyphopichia*, species of the *Yarrowia* clade ferment and assimilate a relatively small number of carbon compounds. Despite this apparent restriction on substrate utilization, the species have been isolated from a variety of habitats. *Y. lipolytica* and *C. galli* show significant lipolytic and proteolytic activity and often occur on lipid and protein rich substrates. *C. incommunis* and *C. bentonensis*, although known only from single isolates, were found in fermenting fruit juices (grape must and cider, respectively), whereas *C. hispaniensis* and *A. aculeatum* are from insects.

The phylogenetic analysis presented here demonstrates no distinction between the genera *Candida* and *Aciculoconidium*. The question of whether *Aciculoconidium* should be maintained as a genus separate from *Candida* will be deferred until additional gene sequences are available for analysis, but phylogenetic studies are demonstrating that, in addition to teleomorphic genera, many of the anamorphic genera are polyphyletic.

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